Study Summary

Assessment of Pubertal Development and Thyroid Function in Juvenile Male and Female Rats

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Project Officer: Kenneth H. Elstein, NHEERL, U.S. EPA

Contractor: TherImmune Research Corporation
15 Firstfield Road
Gaithersburg, MD 20878
Study Director: Meredith S. Rocca

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Technical Monitors: Robert Kavlock, Ph.D., Ralph Cooper, Ph.D.
Earl Gray, Ph.D., Susan Laws, Ph.D., Tammy Stoker, Ph.D.
Jerome Goldman, Ph.D. and Louise Parks, Ph.D.
EDSP Workgroup: Reproductive Toxicology Division
NHEERL, US EPA, Research Triangle Park, NC

Overview of Study: The purpose of this study was to conduct a preliminary validation of two research protocols recommended by EDSTAC for the Tier 1 Endocrine Disruptor Screening Program. Specific goals of the study were: (1) to provide a preliminary validation of the protocols for the Assessment of Pubertal Development and Thyroid Function in Juvenile Male and Female Rats; (2) to assess the robustness of the protocols with regard to intra-laboratory and inter-strain sources of variation; and (3) to provide documentation of the operating procedures required to successfully implement the protocols. The study was conducted under GLP by an independent, commercial laboratory. Chemicals and the dose of each chemical used for testing in the protocols were selected by the U.S. EPA staff based upon published data demonstrating their ability to alter endocrine function in female (ethynyl estradiol, tamoxifen, propylthiouracil,

ketoconazole, pimozide and methoxychlor) and male rodents (methyl testosterone, flutamide, propylthiouracil, ketoconazole, pimozide and dibutylphthalate). The study was conducted in two blocks using Sprague-Dawley and Long Evans ras with six animals/treatment group/block. Vehicle controls (corn oil) were included for each block and strain.

Summary of Study Results and Conclusions: The study was conducted as specified in the Statement of Work and the final report was provided by the contractor on June 30, 2000. In general, the data obtained using the protocols successfully identified the expected endocrine-mediated effects on both male and female pubertal development following exposure to chemicals with estrogenic, anti-estrogenic, androgenic or anti-androgenic activity, inhibitors of steroid and thyroid hormone synthesis, and a dopamine antagonist. A summary of the changes in the major endpoints is shown in Tables 1 and 2. In the female, ethynyl estradiol, tamoxifen (e.g, antagonist and partial estrogen agonist), and methoxychlor advanced the onset of vaginal opening. Propylthiouracil (e.g., an inhibitor of thyroid hormone synthesis), ketoconazole (e.g., an inhibitor of steroid synthesis) or pimozide (e.g., a dopamine antagonist) delayed the age of vaginal opening (Table 1). A comparable measure of the onset of puberty in the male rat, the age of preputial separation, was advanced following exposure to methyl testosterone and delayed by flutamide (e.g., anti-androgen), propylthiouracil, ketoconazole, pimozide or dibuthylphthalate (Table 2).

These data also raised important questions and issues that must be addressed as

the pre-validation of the protocols continues. Specific areas of concern with the actual execution of the protocols include:

(1) The discrepancy between the ages of preputial separation identified in the two strains of rats;

(2) The large degree of variation associated with the means of the fluid-filled and small tissue weights.

In addition, while reviewing this data set, other important issues were raised that should be addressed before further work is conducted. These include the following:

(1) Improving the descriptive text in the protocols such that every key step is clearly described;

(2) Establishing performance criteria for inclusion into the protocols;

(3) Evaluating the lower limits of detection of the protocols by examining dose responses for weaker endocrine disrupting chemicals;

(4) Determining whether or not the protocol should recommend use of a specific strain of rat;

(5) Developing dose selection guidelines (e.g, single or multiple doses; maximum tolerated dose) for chemicals that have a limited toxicological database.

I. Evaluation of Laboratory Performance: TherImmune Research Corporation

A. Control Data and Coefficient of Variance

Two primary concerns were identified while reviewing these data. First, the age of preputial separation occurred later in the Long Evans rats as compared with the Sprague-Dawley. This suggested either a possible strain difference or technical error. Secondly, the variation in the fluid-filled tissues (e.g., seminal vesicles) and smaller tissue

(e.g., pituitary, adrenals, ventral prostate) weights were excessive and indicated possible errors in the performance of these measures.

i. Pubertal indices: There was a marked difference between the strains for the in-life measurement indicative of the onset of puberty in males (i.e., age at preputial separation (PPS)). Although the age at PPS in the control Sprague-Dawley males was within the range expected by comparison to published control values, the age at PPS occurred 2 - 7 days later in the Long Evans controls (Table 3). The age reported for PPS in Block 2 (50.2 ± 2.9 days) was of particular concern, since this advanced age at PPS has never been reported for control males in any strain. Additionally, the coefficients of variation (CV) for the mean PPS in Blocks 1 and 2 were more than 2 -fold higher in the Long Evans rats as compared with the Sprague-Dawley. Discussions with the contractor indicated that for any given day in the study, the same technician recorded the observations in both strains of rats. In addition, the contractor provided the daily observation data along with photographs describing their methods. In those males that were older at PPS, the contractor first noticed a persistent thread of tissue between the glans penis and prepuce. The age of PPS was not recorded until the thread of tissue disappeared. To determine whether or not this might be more prevalent in the Long Evans males, the contractor subsequently submitted PPS data from four additional control groups of Long Evans rats (Table 3). Again, some of these males displayed a persistent thread, and all four of the additional control groups exhibited higher CVs as compared with the control Sprague-Dawley males in Blocks 1 and 2. When the

PPS data from all the Long Evans males were combined, the mean (44.3 ± 3.64 (56)) was closer to the age of PPS that was observed in the Sprague-Dawley, but the CV associated with this mean (e.g, 8.23%) remained greater.

Although these data suggest that the age of PPS in the Long Evans males may be inherently more variable than that in Sprague-Dawley or Wistar rats, additional data are needed to determine if the variability can be replicated by other laboratories, in males from various vendors, and whether or not the use of Long Evans rats should be discouraged in these protocols.

ii. Tissue weights: While reviewing the necropsy data it was noted that the variation in some of the tissue weights was excessive. This was especially true for weights of tissues with fluid-filled lumina (e.g., seminal vesicles and uterus) and the smaller tissues such as the adrenal and pituitary. To put these data in context, we compared the variances associated with these control data with that of control data obtained from a variety of EPA and industrial sources. Tables 4 - 7 compare the coefficients of variation (CV) for the control data from the contractor with historical control data produced by > 8 government and contract laboratories. The CVs for the epididymis, seminal vesicles, adrenal and pituitary weights reported by the contractor were 1.5 - 3 fold higher than those for historical control data from these sources. Through discussions with the contractor, it was learned that because of the number of animals killed at each necropsy, there were delays in weighing the tissues. Thus, some of the smaller tissues and those containing fluid (e.g., seminal

vesicles) may have partially dried prior to weighing. Though experienced laboratories should be cognizant of such problems, this variability could be eliminated in the future by improving the technical description for the dissection and weighing of fluid-filled and small tissues in the written protocol. It is also important to note that the variation in the uterine weights was expected, since uterine weights vary during the estrous cycle and these females were killed on various days of their cycles.

B. Intra-laboratory Sources of Variation

This study was conducted in two complete blocks with a sample size of six animals/treatment/block to evaluate intra-laboratory sources of variation. While in some cases the small sample size may have limited the detection of significant treatment effects in each block, when the data from the blocks were analyzed together the appropriate significant treatment effects were observed. With this in mind, the replication of the study was adequate with the exceptions of the PPS data and tissue weight data discussed in the previous section.

C. Strain Differences

Evaluating two strains of rats in these studies demonstrated that the expected endocrine - mediated changes in pubertal development could be detected in Sprague-Dawley and Long Evans rats. However, the later onset of PPS and greater variance associated with the mean age of PPS in the Long Evans rats as compared with the

Sprague-Dawleys may represent a true strain effect, or one that is attributable to a vendor-associated disparity. While the contractor willingly supplied additional control data sets, this remains an issue for further study, since an increase in variance could potentially produce false negatives (i.e., Type II error) in a screening protocol.

II. Additional Questions/Issues

A. Improving the Descriptive Text in the Protocols

The present study demonstrated the need to improve the descriptive text in the protocols. Feedback from the Contractor indicated areas in the protocols where clarity and more detailed technical direction would have been helpful. Our current aim is to review the protocols to insure that each key step is clearly described. It may also be helpful to develop a manual containing figures and photographs demonstrating the methods for evaluating the pubertal indices, estrous cyclicity and necropsy. In addition, the statistical analysis section needs to be expanded to better describe the options for data analysis (e.g., Delete MANCOVA; Include ANOVA, ANCOVA using necropsy body weight as a covariate, mean tissue weights adjusted for necropsy body weight and/or relative tissue weights (% of BWT), and appropriate tests for heterogeneity of variance).

B. Establishing Performance Criteria

Results from the study conducted by the contractor indicate that there is a need to

incorporate additional laboratory performance criteria into the protocols. Laboratories should be able to demonstrate that they can conduct all technical aspects of the protocol and provide control data that meet acceptable standards consistent with their own historical control data bases as well as data published by other laboratories. For example, the mean ± SD and CVs for all endpoints should certainly fall within the range of published data for the strain of rat used in the study. In addition, the laboratory should be able to demonstrate the ability to detect the expected effects for all endpoints using positive controls. Whether or not acceptable ranges for means, standard deviations and coefficient of variations should be included in the protocol remains an issue for discussion.

The protocols currently do not mandate that positive or negative control groups be included as part of the screening procedure. However, periodically including a positive control group with each chemical tested in the protocol would provide (1) data to verify that the technical aspects of the protocol were performed correctly; and (2) in conjunction with the control data would provide another measure of intra-laboratory variation.

C. Evaluating the Lower Limits of Detection

In the present study the Contractor tested positive controls using doses which maximized the chances of detecting an endocrine-mediated effect. As such, the protocols were successful in identifying the expected endocrine-mediated effects. We recommend that additional dose response data be generated to demonstrate the lower limits of detection of the pubertal protocols for strong and weak endocrine-active chemicals. This

information is critical to define how robust the protocols will be as a screen for identifying endocrine mediated effects.

D. Developing Dose Selection Guidelines

The doses used in this study were selected to maximize the chances of observing a positive effect on pubertal development, and as such, most exceeded the Maximum Tolerated Dose (e.g., MTD is defined as a dose that causes no clinical signs of toxicity and no more than a 10% loss in body weight as compared with the control). As shown in Table 8, the body weights of the rats at necropsy in this study were reduced by 2.3 to 55.9% as compared with their respective controls. It is recognized that such dramatic reductions in body weight would not be appropriate in a screening protocol since it is well documented that lower body weight can delay the onset of puberty in males and females (Goldman et al., 2000; Stoker et al., 2000). The current protocol requires one high dose level "at or just below the MTD". Recent dietary restriction studies by O'Connor et al. (1999, 2000) have shown that neither organ weights or serum hormone concentrations are adversely altered in adult males treated for 15 days when body weight does not deviate more than 10% from the control group fed *ad libitum*. In addition, two recent studies by Stoker et al. (2000) and Laws et al. (2000) have monitored the effects of reduced body weight on pubertal development in male and female Wistar rats. In these studies, necropsy body weight in food-restricted males and females were reduced by 14% and 12%, respectively, as compared with the controls fed *ad libitum*. Preputial separation was significantly delayed by 2 days in the food-restricted males as compared with the

controls fed *ad libitum*. However, while the age of vaginal opening in the females was delayed by 1.6 days, this was not significantly different from the controls fed *ad libitum*. Additional data are needed to determine the effect of reduced body weight on all the endpoints in the pubertal protocols.

Another possible caveat in selecting a dose based upon the MTD is that estrogen is a known anorexic in rats (e.g., Reynolds and Bryson, 1974). Although, the effect of estrogen on food intake in prepubertal females has not been clearly defined, it is reasonable to assume that a reduction in body weight may occur if a test chemical is estrogenic. If such a reduction in body weight is mistaken for systemic toxicity and the dosage selected for the protocol reduced, then selecting a dose based upon the standard MTD criteria may produce false negatives (e.g, since food intake is estrogen-dependent, a dose would be selected that is too low to demonstrate an endocrine-mediated effect in the protocol). Indeed, the data from the ethynyl estradiol-treated females in these studies demonstrate a reduction of 5.9% (Sprague-Dawley) and 11.5% (Long Evans) in necropsy body weight as compared with the controls. A detailed analysis of the changes in body weight is currently being conducted to determine if greater differences in the body weights of the controls and ethynyl estradiol-treated females occurred during the 20-day treatment period. Additional dose response data are needed to accurately determine the effects of estrogen and estrogenic test chemicals on food intake and body weight in the prepubertal females before the traditional MTD can be regarded as the appropriate guide for dose selection.

A final point about dose selection for the protocol is that identifying a single dose

based upon the MTD may not be a simple endeavor for chemicals that have a limited toxicological database. Therefore, in some cases it may be preferable to include a dose response. Whether or not a single or multiple doses should be recommended in the protocols is an issue that warrants further discussion.

E. Evaluating Whether or Not a Specific Strain Should be Recommended

Whether or not a particular strain of rat should be recommended for testing in the protocol needs further consideration. While the ideal protocol would be capable of identifying endocrine-active compounds in any strain of rat, we currently do not have the data to be certain that this will be the case. For example, if the greater variation associated with the age at preputial separation is indeed a problem inherent to Long Evans males, then such a degree of variation may result in false negatives. The fact that subtle differences in metabolism may exist between some strains may also prove to be problematic. For further pre-validation studies it may be prudent to limit the strain to Sprague-Dawley rats since they have been used extensively in the United States for toxicological assessment, and ample historical control data are available. However, to determine how robust the protocols are as screens for detecting endocrine-active chemicals, the issues associated with possible strain differences must be resolved.

III. Suggestions for Additional Pre-validation Studies

To address the questions and issues discussed in this document, two specific studies are recommended:

-Dose response studies to evaluate the lower limits of detection of the protocols using strong and weak endocrine-active chemicals. The chemicals tested would be identical to those used in the pubertal studies (e.g., an estrogen agonist and antagonist, an androgen agonist and antagonist, inhibitors of steroid and thyroid hormone synthesis, and a dopamine antagonist),

- A study to characterize the effects of reduced food intake and body weight on the endpoints of the female and male protocols. This would include dose responses with food-restricted controls, ethynyl estradiol and a weakly estrogenic environmental chemical.

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Table 1. Summary of Significant Effects on Major Endpoints in Female Sprague-Dawley and Long Evans Rats

Treatment	Mode of Action	Age at Vaginal Opening[a]	Age at Estrus[a]	Histopathology[a]	TSH[a]	T4[a]
Etynyl estradiol (0.005 mg/kg/d)	ER agonist	↓	↓	✓	-	-
Tamoxifen (10 mg/kg/d	ER antagonist, Partial agonist	↓	↑	✓	↑ SD	↑
Propylthiouracil (240 mg/kg/d)	Inhibitor of T4 Synthesis	↑ SD	↑ SD	✓	↑	↓
Ketoconazole (100 mg/kg/d)	Inhibits Steroidogenesis	↑ SD	↑	✓	-	↓
Pimozide (30 mg/kg/d)	Dopamine receptor antagonist	↑	-	✓	↓ LE	↓
Methoxychlor (100 mg/kg/d)	ER agonist	↓	↓	✓	↓ LE	-

Key: ↓= Significantly decreased compared to control LE = Long Evans rats only E = Age at first estrus
↑= Significantly increased compared to control SD = Sprague-Dawley rats only ✓=Affected histopathology
ER=Estrogen receptor

[a]Data are consistent with expected results for each mode of action.

Table 2. Summary of Significant Effects on Major Endpoints in Male Sprague-Dawley and Long Evans Rats

Treatment	Mode of Action	Age at Preputial Separation [a]	Histopathology [a]	TSH [a]	T4[a]
Flutamide (50 mg/kg/d)	AR antagonist	↑	✓	-	-
Methyl Testosterone (80 mg/kg/d)	AR agonist	↓	✓	-	-
Propylthiouracil (240 mg/kg/d)	Inhibitor of T4 Synthesis	↑	✓	↑	↓
Ketoconazole (100 mg/kg/d)	Inhibits Steroidogenesis	↑	-	-	-
Pimozide (30 mg/kg/d)	Dopamine receptor antagonist	↑	✓LE	-	-
Dibutylphthalate (1000 mg/kg/d)	Anti-androgenic (not AR mediated)	↑LE	✓LE	-	↓LE

Key: ↓= Significantly decreased compared to control LE = Long Evans rats only E = Age at first estrus
↑= Significantly increased compared to control SD = Sprague-Dawley rats only ✓=Affected histopathology
AR=Androgen receptor

[a]Data are consistent with expected results for each mode of action.

Table 3. Strain Comparison of Preputial Separation (PPS) In Control Groups (TherImmune Data)

Strain	Block	Age (Days) at Preputial Separation (Mean ± SD (n))	Coefficient of Variation (%)
Sprague-Dawley	1	43.0 ± 1.10 (6)	2.56
	2	43.0 ± 0.91 (6)	2.12
Long Evans	1	44.8 ± 2.23 (6)	4.98
	2	50.2 ± 2.92 (6)	5.82
Long Evans (Additional controls)	3	42.2 ± 1.55 (11)	3.67
	4	42.6 ± 1.85 (11)	4.34
	5	43.8 ± 3.78 (11)	8.63
	6	45.0 ± 4.04 (11)	8.97

Table 4. Comparison of TherImmune Data with Historical Data for Age at Vaginal Opening in Control Sprague-Dawley, Wistar and Long Evans Rats.

Strain	Contract and Government Laboratory							
	Contract Lab. 1	Contract Lab. 2	Contract Lab. 3	Contract Lab. 4	Contract Lab. 5	Contract Lab. 6	EPA Lab. 1	TherImmune
Sprague-Dawley	30.9 ± 1.5	31.4 ± 1.1	31.8 ± 0.69	32.0 ± 1.5	33.6 ± 2.3	33.1 ± 2.6	32.7 ± 1.1	34.9 ± 1.3
	CV= 4.85%	3.50%	2.17%	4.69%	6.85%	7.85%	3.36%	3.75%
	Contract Lab. 7	Contract Lab. 7	Contract Lab. 7	Contract Lab. 7	EPA Lab. 2	EPA Lab. 2	EPA Lab. 2	
Wistar	34.3 ± 1.3	34.1 ± 1.3	33.7 ± 0.97	35.4 ± 1.8	32.8 ± 2.0	32.4 ± 2.1	33.1 ± 1.2	
	CV= 3.79%	3.82%	2.88%	5.08%	6.14%	6.38%	3.74%	
	EPA Lab. 2	EPA Lab. 2	EPA Lab. 2	EPA Lab. 2				TherImmune
Long Evans	33.4 ± 1.8	32.25 ± 2.0	33.7 ± 1.5	30.6 ± 1.2				36.3 ± 2.1
	CV= 5.39%	6.36%	4.39%	3.89%				5.67%

Data are reported as Mean ± SD; CV= Coefficient of variation

Table 5. Comparison of TherImmune Data with Historical Data for Age at Preputial Separation in Control Sprague-Dawley, Wistar and Long Evans Rats.

Strain	Contract and Government Laboratory							
	Contract Lab. 1	Contract Lab. 2	Contract Lab. 3	Contract Lab. 4	Contract Lab. 5	Contract Lab. 6	Contract Lab. 6	TherImmune
Sprague-Dawley	43.5 ± 1.5	44.0 ± 2.5	44.9 ± 0.98	49.4 ± 3.5	43.8 ± 2.3	42.1 ± 1.0	42.6 ± 1.3	43.0 ± 1.0
	CV= 3.63%	5.68%	2.18%	7.09%	5.41%	2.38%	3.05%	2.33%
	Contract Lab. 7	Contract Lab. 7	Contract Lab. 7	Contract Lab. 7	EPA Lab. 3	EPA Lab. 3		
Wistar	44.8 ± 1.3	44.9 ± 1.4	44.7 ± 3.5	45.2 ± 1.5	41.9 ± 0.78	42.75 ± 0.04		
	CV= 2.79%	3.18%	7.81%	3.32%	1.87%	0.11%		
	EPA Lab. 4							TherImmune
Long Evans	40.9 ± 1.9							47.5 ± 3.7
	CV= 4.70%							7.85%

Data are reported as Mean ± SD; CV= Coefficient of variation

Table 6. Comparison of TherImmune Data with Historical Data for Necropsy Body Weight and Tissue Weights in Control Sprague-Dawley, Wistar and Long Evans Female Rats.

	Contract or Government Laboratory						
	Contract Lab. 5	Contract Lab. 5	Contract Lab. 2	EPA Lab. 2	EPA Lab. 2	TherImmune	TherImmune
Strain	Sprague-Dawley	Sprague-Dawley	Wistar	Wistar	Wistar	Sprague-Dawley	Long Evans
Body Weight(g)		387 ± 30	150 ± 8.7	140 ± 7.7	303 ± 20	146 ± 8.0	161 ± 8.8
		CV= 7.75%	CV= 5.79%	CV= 5.49%	CV= 6.60%	CV= 5.45%	CV= 5.49%
Liver (g)	12.2 ± 1.54	17.6 ± 2.3	6.73 ± 0.80	6.09 ± 0.53		6.06 ± 0.45	6.96 ± 0.72
	12.6%	13.1%	11.8%	8.74%		7.43%	10.3%
Kidney (g)	2.36 ± 0.29	2.73 ± 0.27	1.42 ± 0.12	1.33 ± 0.09		1.23 ± 0.09	1.45 ± 0.13
	12.2%	9.89%	8.58%	7.32%		7.32%	8.97%
Uterus + fluid (g)		0.53 ± 0.09*	0.354± 0.205	0.346± 0.231	0.525 ± 0.09*	0.29 ± 0.15	0.27 ± 0.11
		16.9%	57.9%	66.8%	17.7%	51.7%	40.7%
Ovary (g)	0.141 ± 0.019	0.109 ± 0.017	0.072 ± 0.019	0.072 ± 0.019	0.061 ± 0.011	0.065 ± 0.013	0.082± 0.017
	13.5%	15.6%	26.4%	26.4%	18.0%	20.0%	20.7%
Adrenals (g)		0.066 ± 0.008	0.045 ± 0.005	0.038 ± 0.005		0.043 ± 0.008	0.037 ± 0.007
		12.1%	11.1%	13.2%		18.6%	18.9%
Pituitary (g)	0.016 ± 0.003	0.013 ± 0.020	0.0079±0.0008	0.0076±0.0007		0.007 ± 0.002	0.007 ± 0.003
	18.7%	15.4%	10.1%	9.2%		28.6%	42.9%

Data are reported as Mean ± SD; CV= Coefficient of variation; * All females in these groups were killed during diestrus
Note: Ages of females at necropsy varied between studies

Table 7. Comparison of TherImmune Data with Historical Data for Necropsy Body Weight and Tissue Weights in Control Sprague-Dawley, Wistar and Long Evans Male Rats.

	Contract or Government Laboratory						
	Contract Lab. 4	Contract Lab. 5	Contract Lab. 6	EPA Lab. 3	EPA Lab. 4	TherImmune	TherImmune
Strain	Sprague-Dawley	Sprague-Dawley	Wistar	Wistar	Sprague-Dawley	Sprague-Dawley	Long Evans
Body Weight(g)	500 ± 53	558 ± 50	616 ± 64		313 ± 22	258 ± 12	285 ± 21
	CV= 10.5%	CV= 9.0%	CV= 10.4%		CV= 7.1%	CV= 4.97%	CV= 7.15%
Testes (g)	1.81± 0.19 *	3.73 ± 0.27 **	4.15 ± 0.34 **	1.40 ± 0.04 *	2.88 ± 0.16 **	3.09 ± 0.14 **	2.67 ± 0.14 **
	10.6%	7.3%	8.2%	3.0%	5.59%	4.53%	5.24%
Epididymis (g)	0.60 ± 0.07 *	1.41 ± 0.12 **	1.50 ± 0.14 **	0.23 ± 0.007 *	0.26 ± 0.02 *	0.49 ± 0.039 **	0.48 ± 0.075 **
	11.1%	8.58%	8.97%	3.23%	7.9%	7.93%	15.5%
Sem. Vesicles + fluid (g)			2.07± 0.34	0.557± 0.037	0.368 ± 0.053	0.477± 0.15	0.371 ± 0.12
			16.4%	6.8%	14.6%	31.4 %	32.4%
VentralProstate (g)			0.87 ± 0.21	0.265 ± 0.011	0.236 ± 0.028	0.221 ± 0.027	0.182± 0.062
			24.1%	4.4%	12.2%	12.2%	34.1%
Adrenals (g)			0.059 ± 0.007		0.050 ± 0.006	0.0446±0.0086	0.0464±0.0088
			11.9%		13.2%	19.3%	18.9%
Pituitary (g)		0.014 ± 0.002	0.013 ± 0.002	0.0076±0.0003	0.0094±0.0004	0.0086±0.0011	0.0074±0.0023
		14.3%	15.4%	4.37%	4.0%	12.8%	31.1%

Data are reported as Mean ± SD; CV= Coefficient of variation; Note: Ages of males at necropsy varied between studies
* Indicates that one testis or epididymis was weighed; ** Indicates that two testes or epididymis were weighed

Table 8. Body Weight Loss (% Lower than Control) at Necropsy (Therimmune Data).

	Treatment Groups											
	Ethynyl Estradiol (0.005 mg/kg)		Tamoxifen (10 mg/kg)		Propylthiouracil (240 mg/kg)		Ketoconazole (100 mg/kg)		Pimozide (30 mg/kg)		Methoxychlor (100 mg/kg)	
	Block 1	2	1	2	1	2	1	2	1	2	1	2
Sprague-Dawley[A]	6.0	5.8	17.7	20.8	32.1	33.7	2.3	5.2	12.2	13.7	6.4	4.8
Long Evans[B] Females	12.5	10.5	21.6	21.4	45.1	48.2	8.9	13.5	24.9	31.2	14.6	11.3

	Flutamide (50 mg/kg)		Methyl Testosterone (80 mg/kg)		Propylthiouracil (240 mg/kg)		Ketoconazole (100 mg/kg)		Pimozide (30 mg/kg)		Dibutylphthalate (1000 mg/kg)	
	Block	2	1	2	1	2	1	2	1	2	1	2
Sprague-Dawley[C]	1.5	6.7	6.0	10.9	55.9	53.7	5.8	10.1	14.7	20.4	5.1	6.7
Long Evans[D] Males	11.3	9.6	10.4	9.8	62.4	61.8	13.3	6.8	25.3	22.5	10.8	16.1

[A]Control Body weight at necropsy: (SD Females) Block 1 (147.5 ± 4.3 (6); Block 2 (146.5 ± 2.2 (6).
[B]Control Body weight at necropsy: (LE Females) Block 1 (164.7 ± 2.7 (6); Block 2 (158.6 ± 4.2 (6).
[C]Control Body weight at necropsy: (SD Males) Block 1 (259.1 ± 3.0 (6); Block 2 (258.0 ± 7.2 (6).
[D]Control Body weight at necropsy: (LE Males) Block 1 (292.3 ± 6.4 (6); Block 2 (275.5 ± 10.3 (5).